

464.1001

being produced by a unique reaction series composed of N reaction steps, wherein each compound is prepared from a component, and N is an integer from at least 1 to about 100, which comprises:

- a) dividing a population of solid supports having at least one type of a functional group at the surface of said solid support selected from the group consisting of CO_2H , OH , SH , NH_2 , NHR , CH_2Cl , CH_2Br and CHN_2 , wherein R is a linear $\text{C}_1\text{-C}_9$ alkyl group, into M batches, where M is an integer from at least 2 to about 25;
- b) coupling the M batches of solid support in a set of at least one reaction respectively with M different components so as to form a bond with the solid support via said functional group, said components being independently protected or unprotected;
- c) adding to each batch, prior to coupling step b), concurrently therewith, or subsequently to step b), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each component and capable of forming a bond to the solid support or to the component, wherein said fluorophore tag represents a bit of a binary code, and comprises zero, one or more than one fluorescent dye, multiple fluorescent dyes, said dye(s) being spectrally distinguishable by excitation wavelength, emission wavelength, excited-state lifetime or emission intensity, the emission intensity being distinguishable by adjusting the ratio of the relative quantities of each fluorophore; and either ?
- d) recombining all M batches, said recombining step being either prior to or subsequent to step c), and steps e)-g); or
- e) performing an assay capable of indicating that any compound in the library either while bound to or cleaved from its solid support has the property of interest;
- f) collecting spectral fluorescence data for each respective solid support so as to determine respective relative abundance of the fluorophore tags bound thereto; and ?

464.1001

- g) analyzing the collected spectral fluorescence data by comparing the respective relative abundances of the fluorophore tags determined in step f) so as to determine the unique reaction series for the component, thereby identifying the compound having the property of interest.

75.

The method of claim 74, wherein the dye(s) are spectrally distinguishable by emission intensity, the emission intensity being distinguishable by adjusting the ratio of the relative quantities of each fluorophore.

76.

The method of claim 74, wherein the fluorophore tags are dyes selected from the group consisting of compounds with the chemical names:

3-(ϵ -carboxypentyl)-3'-ethyl-oxacarbocyanine-6,6'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethyl-3H-benz(e)indocarbocyanine-5,5',7,7'-tetrasulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethyl-3H-benz(e)indodicarbocyanine-5,5',7,7'-tetrasulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3,3',3'-tetramethylindotricarbocyanine-5,5'-disulfonic acid

and are activated as active esters selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

464.1001

77. The method of claim 74, wherein the fluorophore tags are dyes selected from the group consisting of compounds with the chemical names:

6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino) hexanoic acid

6-((4,4-difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl) amino) hexanoic acid,

6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-2-propionyl) amino)hexanoic acid,

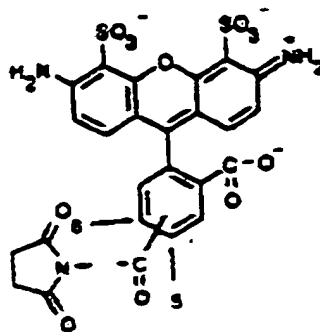
6-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl) phenoxy) acetyl) amino)hexanoic acid,

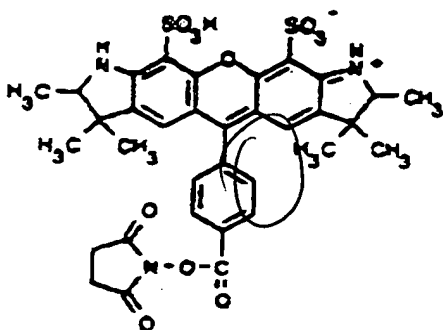
6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl) styryloxy)acetyl) aminohexanoic acid, and

6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl) styryloxy) acetyl)aminohexanoic acid,

and are activated as active esters selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

78. The method of claim 74, wherein the fluorophore tags are dyes selected from the group consisting of compounds with the chemical structures:

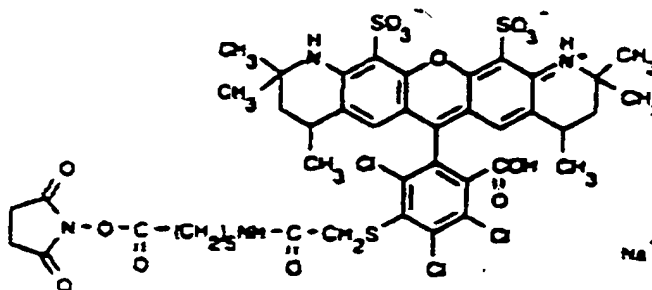




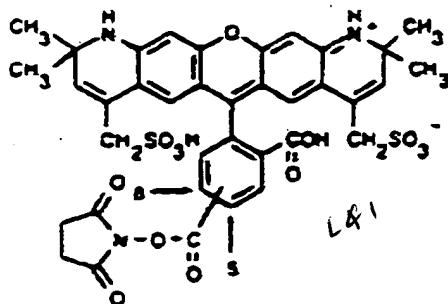
464.1001

L39

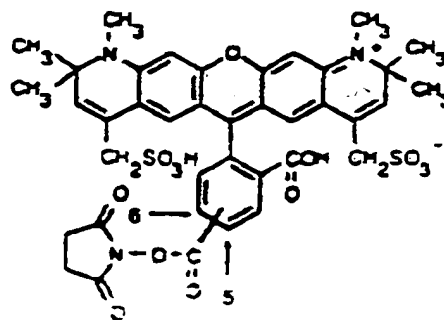
240



L42



L41



L41

Original
claim 23 (79.)

The method of claim 74, wherein the fluorescence data are collected from multiple solid supports using multi-spectral imaging methods.

80. The method of claim 74, wherein the solid support is a polymeric bead, and spectral fluorescence data is collected by:

a) forming either a static planar array or a dynamic planar array of beads; and

electrode
5

464.1001

b) obtaining a fluorescence image for each bead.

81. The method of claim 80, wherein the planar array of beads is formed adjacent to the planar walls of a sandwich flow cell and controlled by light-controlled electrokinetic means.

not used 82. The method of claim 80, wherein the planar array of beads is formed by using an apparatus capable of dynamically assembling and disassembling an array of beads at an interface between an electrode and an electrolyte solution, said apparatus comprising:

- i) an electrode, an electrolyte solution and an interface therebetween
- ii) a plurality of beads located in said electrolyte solution;
- iii) said electrode being patterned to include at least one area of modified electrochemical properties;
- iv) an illumination source which illuminates said electrode with a predetermined light pattern;
- v) an electric field generator which generates an electric field at said interface to cause the assembly of an array of beads in accordance with the predetermined light pattern and the electrochemical properties of said electrode; and
- vi) an electric field removal unit which removes said electric field to cause the disassembling of said array of beads.

83. The method of 80, wherein spectral fluorescence data are collected for the bead array by initially forming a spatially encoded array of beads being produced by a unique reaction series composed of N coupling or reaction steps, wherein each compound is prepared from components which are independently the same or different, and N is an integer from at least 1 to about 100, which comprises:

- a) dividing a population of solid supports having at least one type of a first functional group at the surface of said solid support selected from the group consisting of

464.1001

CO₂H, OH, SH, NH₂, NHR, CH₂Cl, CH₂Br and CHN₂, wherein R is a linear C₁-C₉ alkyl group, into *M* batches, where *M* is an integer from at least 2 to about 50;

- b) coupling the *M* batches of solid support in a set of at least one reaction respectively with *M* different initial components so as to form a bond with the solid support via said functional group, said components being protected or unprotected at a group which is capable of participating in a further coupling step and orthogonally protected at non-participating group(s);
- c) adding to each batch, optionally prior to coupling step b), concurrently therewith, or subsequently to step b), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each initial component or a reaction step b), said tag being identified by its characteristic excitation wavelength(s), emission wavelength(s), excited state lifetime and emission intensity, said tag being activated so as to be capable of forming either a direct bond to the surface of the solid support, optionally via a second functional group which is optionally protected and may be the same or different from said first functional group, a direct bond to the initial component which if protected is priorly deprotected, or an indirect bond via a C₁-C₉ linear or branched alkyl moiety which is optionally interrupted by at least one oxygen or nitrogen atom or a carbonyl, (C=O)NH or NH(C=O) moiety, said linker being bonded to said first functional group at the surface of the solid support, wherein when said second functional group is protected, said second functional group is deprotected prior to forming said direct or indirect bond;
- d) optionally recombining all *M* batches and cleaving any protecting group present at a group which is to participate in a further coupling step, said recombining step optionally being subsequent to step e);
- e) iteratively *N*-1 times
maintaining each of said *m* × *n* bead arrays in one of the corresponding *m* × *n* compartments.

464.1001

84. The method of claim 83, wherein said compartments are hydrophilic and the remainder of said electrode surface is hydrophobic.

85. An apparatus for identifying a compound having a selected property of interest in a library of compounds, each of said compounds being bound to its respective solid support, and being produced by a unique reaction series composed of N reaction steps, wherein each compound is prepared from a component, and N is an integer from at least 1 to about 100, which comprises:

- a) an electrode and an electrolyte solution having an interface therebetween,
- b) an electric field generator which generates an electric field at an interface between an electrode and an electrolyte solution;
- c) said electrode being patterned to modify the electrochemical properties of said electrode;
- d) an illuminating source which illuminates said interface with a predetermined light pattern to control the movement of said particles in accordance with said predetermined light pattern and the electrochemical properties of said electrode;
- e) means for preparing said chemical library, which comprises:
 - i) means for dividing a population of solid supports having at least one type of a functional group at the surface of said solid support selected from the group consisting of CO_2H , OH , SH , NH , NHR , CH_2Cl , CH_2Br and CHN_2 , wherein R is a linear C_1 - C_9 alkyl group, into M batches, where M is an integer from at least 2 to about 25;
 - ii) means for coupling the M batches of solid support in a set of at least one reaction respectively with M different components so as to form a bond with the solid support via said functional group, said components being independently protected or unprotected;

464.1001

- iii) means for adding to each batch, prior to coupling step ii), concurrently therewith, or subsequently to step ii), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each component and capable of forming a bond to the solid support or to the component, which if protected is priorly deprotected; and either
- iv) means for recombining all M batches, said recombining step being either prior to or subsequent to step v), and steps v)-vii); or
- v) means for performing an assay capable of indicating that any compound in the library either while bound to or cleaved from its solid support has the property of interest;
- vi) means for collecting spectral fluorescence data for each respective solid support so as to determine respective relative abundance of the fluorophore tags bound thereto; and
- vii) means for analyzing the collected spectral fluorescence data by comparing the respective relative abundances of the fluorophore tags determined in step vi) so as to determine the unique reaction series for the component, thereby identifying the compound having the property of interest.

86. A method of identifying a compound having a selected property of interest in a library of compounds, each of said compounds being bound to its respective solid support, and being produced by a unique reaction series composed of N coupling or reaction steps, wherein each compound is prepared from components, which are independently the same or different, and N is an integer from at least 1 to about 100, which comprises:

- a) dividing a population of solid supports having at least one type of a functional group at the surface of said solid support selected from the group consisting of CO_2H , OH , SH , NH_2 , NHR , CH_2Cl , CH_2Br and CHN_2 , wherein R is a linear $\text{C}_1\text{-C}_9$ alkyl group, into M batches, where M is an integer from at least 2 to about 50;

464.1001

- b) coupling the M batches of solid support in a set of at least one reaction respectively with M different initial components so as to form a bond with the solid support via said functional group, said components being protected or unprotected at a group which is capable of participating in a further coupling step and orthogonally protected at non-participating group(s);
- c) adding to each batch, prior to coupling step b), concurrently therewith, or subsequently to step b), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each initial component or a reaction step b) and capable of forming a bond to the solid support or to the initial component, wherein said fluorophore tag represents a bit of a binary code, and comprises zero, one or more than one fluorescent dye, multiple fluorescent dyes, said dye(s) being spectrally distinguishable by excitation wavelength, emission wavelength, excited-state lifetime or emission intensity, the emission intensity being distinguishable by adjusting the ratio of the relative quantities of each fluorophore ; and either
- d) recombining all M batches and cleaving any protecting group present at a group which is to participate in a further coupling step, said recombining step being either prior to or subsequent to step e), and steps e)-h); or
- e) iteratively $N-1$ times
 - (1) dividing a population of solid supports into $M(N)$ batches, wherein $M(N)$ depends on N and is an integer from at least 2 to about 25;
 - (2) coupling the $M(N)$ batches of solid support respectively with $M(N)$ different components, wherein $M(N)$ is the number of batches during the N th step, said components being protected or not protected at a group which is capable of participating in a further coupling step and orthogonally protected at a nonparticipating group(s);
 - (3) adding to each batch either prior to coupling step (2), concurrently therewith, or subsequently to step (2), from about 0.001 to about 0.5 molar

464.1001

equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each component in the N th coupling step (2) and capable of forming a bond to the solid support or to the $(N-1)$ th component, wherein said fluorophore tag represents a bit of binary code, and comprises zero, one or more than one fluorescent dye, multiple fluorescent dyes, said dye(s) being spectrally distinguishable by excitation wavelength, emission wavelength, excited-state lifetime or emission intensity, the emission intensity being distinguishable by adjusting the ratio of the relative quantities of each fluorophore;

- (4) recombining all $M(N)$ batches and cleaving any protecting group present at a group which is to participate in a further coupling step; as to form a compound having N components;
 - (f) performing an assay capable of indicating that any compound in the library either while bound to or cleaved from its solid support has the property of interest;
 - (g) collecting spectral fluorescence data for each respective solid support so as to determine respective relative abundances of the fluorophore tags bound thereto; and
 - (h) analyzing the collected spectral fluorescence data by comparing the respective relative abundances of the fluorophore tags determined in step g) so as to determine the N components coupled in the unique reaction series for the component, thereby identifying the compound having the property of interest.
87. The method of claim 86, wherein the dye(s) are spectrally distinguishable by emission intensity, the emission intensity being distinguishable by adjusting the ratio of the relative quantities of each fluorophore
88. The method of claim 86, wherein the ratio is 1:1, 2:1, 3:1 or 4:1.

464.1001

89. The method of claim 86, wherein the fluorophore tags are dyes selected from the group consisting of compounds with the chemical names :

3-(ϵ -carboxypentyl)-3'-ethyl-oxacarbocyanine-6,6'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3',3'-tetramethyl-3H-benz(e)indocarbocyanine-5,5',7,7'-tetrasulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3',3'-tetramethyl-3H-benz(e)indodicarbocyanine-5,5',7,7'-tetrasulfonic acid

1-(ϵ -carboxypentyl)-1'-ethyl-3,3',3'-tetramethylindotricarbocyanine-5,5'-disulfonic acid

and are activated as active esters selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

90. The method of claim 86, wherein the fluorophore tags are dyes selected from the group consisting of compounds with the chemical names:

6-((4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino) hexanoic acid

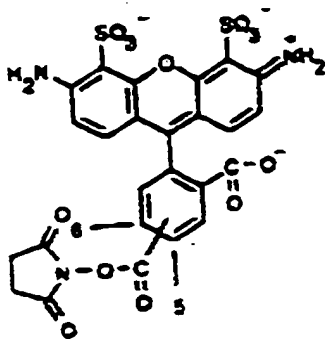
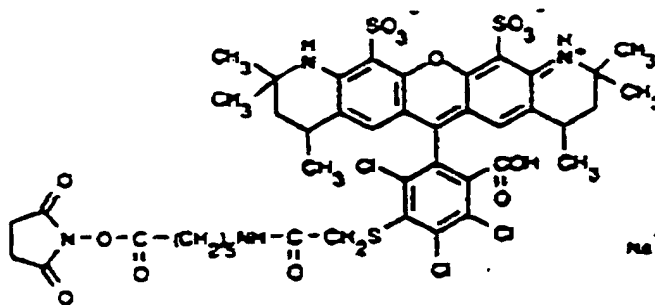
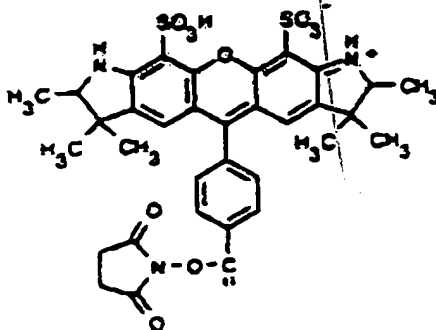
6-((4,4-difluoro-5-phenyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)amino) hexanoic acid,

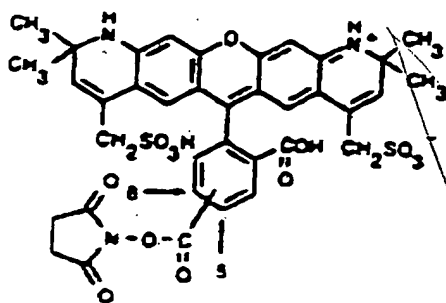
464.1001

6-(((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-2-propionyl) amino)hexanoic acid,
 6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)phenoxy) acetyl) amino)hexanoic acid,
 6-(((4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy)acetyl) amino)hexanoic acid, and
 6-(((4,4-difluoro-5-(2-pyrrolyl)-4-bora-3a,4a-diaza-s-indacene-3-yl)styryloxy) acetyl)amino)hexanoic acid,

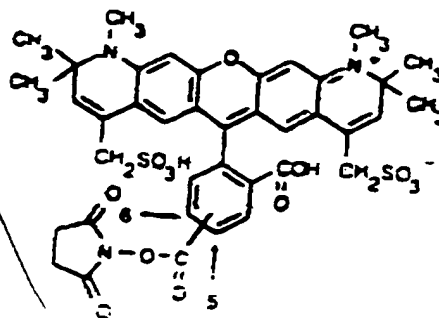
and are activated as active esters selected from the group consisting of succinimidyl, sulfosuccinimidyl, p-nitrophenol, pentafluorophenol, HOBt and N-hydroxypiperidyl.

91. The method of claim 86, wherein the fluorophore tag are dyes selected from the group consisting of compounds with the chemical structures:

2 Li⁺Na⁺



464.1001



92. The method of claim 86, wherein the solid support is a bead, and spectral fluorescence data are collected by
- forming either a static planar array or a ~~dynamic planar~~ ^{electro}array of beads; and
 - obtaining a fluorescence image for at least one bead.
93. The method of claim 92, wherein the planar array of beads is formed adjacent to the planar walls of a sandwich flow cell and controlled by light-controlled electrokinetic means.
- nonelected* 94. The method of claim 92, wherein the planar array of beads is formed by using an apparatus capable of dynamically assembling and disassembling an array of beads at an interface between an electrode and an electrolyte solution, said apparatus comprising:
- an electrode, an electrolyte solution and an interface therebetween
 - a plurality of beads located in said electrolyte solution;
 - said electrode being patterned to include at least one area of modified electrochemical properties;
 - an illumination source which illuminates said electrode with a predetermined light pattern;
 - an electric field generator which generates an electric field at said interface to cause the assembly of an array of beads in accordance with the predetermined light

464.1001

- pattern and the electrochemical properties of said electrode; and
- vi) an electric field removal unit which removes said electric field to cause the dissembling of said array of beads.

95. The method of claim 92, wherein spectral fluorescence data are collected for the bead array by initially forming a spatially encoded array of beads suspended at an interface between an electrode and an electrolyte solution, comprising the following steps:

- i) providing an electrode and an electrolyte solution;
- ii) providing multiple types of particles, each type being stored in accordance with chemically or physically distinguishable particle characteristics in one of a plurality of reservoirs, each reservoir containing a plurality of like-type particles suspended in said electrolyte solution;
- iii) providing said reservoirs in the form of an $m \times n$ grid arrangement;
- iv) patterning said electrode to define $m \times n$ compartments corresponding to said $m \times n$ grid of reservoirs;
- v) depositing $m \times n$ droplets from said $m \times n$ reservoirs onto said corresponding $m \times n$ compartments, each said droplet originating from one of said reservoirs and remaining confined to one of said $m \times n$ compartments and each said droplet containing at least one particle;
- vi) positioning a top electrode above said droplets so as to simultaneously contact each said droplet;
- vii) generating an electric field between said top electrode and said $m \times n$ droplets;
- viii) using said electric field to form a bead array in each of said $m \times n$ compartments, each said bead array remaining spatially confined to one of said $m \times n$ droplets;
- ix) illuminating said $m \times n$ compartments on said patterned electrode with a predetermined light pattern to maintain the position of said bead arrays in accordance with said predetermined light pattern and the pattern of $m \times n$ compartments; and

464.1001

- x) positioning said top electrode closer to said electrode thereby fusing said $m \times n$ droplets into a continuous liquid phase, while maintaining each of said $m \times n$ bead arrays in one of the corresponding $m \times n$ compartments.

96. The method of claim 95, wherein said compartments are hydrophilic and the remainder of said electrode surface is hydrophobic.

97. An apparatus for identifying a compound having a selected property of interest in a library of compounds, each of said compounds being bound to its respective solid support, and being produced by a unique reaction series composed of N reaction steps, wherein each compound is prepared from a set of components which are independently the same or different, and N is an integer from at least 1 to about 100, said solid support being at least one particle array, said apparatus comprising:

- a) an electrode and an electrolyte solution having an interface therebetween,
- b) an electric field generator which generates an electric field at an interface between an electrode and an electrolyte solution;
- c) said electrode being patterned to modify the electrochemical properties of said electrode;
- d) an illuminating source which illuminates said interface with a predetermined light pattern to control the movement of said particles in accordance with said predetermined light pattern and the electrochemical properties of said electrode;
- e) means for preparing said chemical library, which comprises:
 - i) means for dividing a population of solid supports having at least one type of a functional group at the surface of said solid support selected from the group consisting of CO_2H , OH , SH , NH_2 , NHR , CH_2Cl , CH_2Br and CHN_2 , wherein R is a linear C_1 - C_9 alkyl group, into M batches, where M is an integer from at least 2 to about 50;
 - ii) means for coupling the M batches of solid support in a set of at least one

464.1001

reaction respectively with M different initial components so as to form a bond with the solid support via said functional group, said components being independently protected or unprotected at a group which is to participate in a further coupling step and orthogonally protected at non-participating group(s);

- iii) means for adding to each batch, either prior to coupling step ii), concurrently therewith, or subsequently to step ii), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag associated uniquely with each initial component and capable of forming a bond to the solid support or to the component, said tag being identified by its characteristic excitation wavelength(s), emission wavelength(s), excited state lifetime and emission intensity, and either
- iv) means for recombining all M batches and cleaving any protecting group present at a group which is to participate in a further coupling step, said recombining step, and steps v)-viii); or
- vi) means for iteratively $N-1$ times
 - (1) dividing a population of solid supports into $M(N)$ batches, wherein $M(N)$ depends on N and is an integer from at least 2 to about 25;
 - (2) coupling the $M(N)$ batches of solid support respectively with $M(N)$ different components, wherein $M(N)$ is the number of batches during the N th step, said components being protected or not protected at a group which is capable of participating in a further coupling step and orthogonally protected at a nonparticipating group(s);
 - (3) adding to each batch either prior to coupling step (2), concurrently therewith, or subsequently to step (2), from about 0.001 to about 0.5 molar equivalent of a spectrally distinguishable fluorophore tag